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Research report

Ultrastructural features and synaptic connections of hilar ectopic granule cells in the rat dentate gyrus are different from those of granule cells in the granule cell layer

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Abstract

Several investigators have shown the existence of dentate granule cells in ectopic locations within the hilus and molecular layer using both Golgi and retrograde tracing studies but the ultrastructural features and synaptic connections of ectopic granule cells were not previously examined. In the present study, the biocytin retrograde tracing technique was used to label ectopic granule cells following injections into stratum lucidum of CA3b of hippocampal slices obtained from epileptic rats. Electron microscopy was used to study hilar ectopic granule cells that were located 20–40 μm from the granule cell layer (GCL). They had ultrastructural features similar to those of granule cells in the GCL but showed differences, including nuclei that often displayed infoldings and thicker apical dendrites. At their origin, these dendrites were 6 μm in diameter and they tapered down to 2 μm at the border with the GCL. Both biocytin-labeled and unlabeled axon terminals formed exclusively asymmetric synapses with the somata and proximal dendrites of hilar ectopic granule cells. The mean number of axosomatic synapses for these cells was three times that for granule cells in the GCL. Together, these data indicate that hilar ectopic granule cells are postsynaptic to mossy fibers and have less inhibitory input on their somata and proximal dendrites than granule cells in the GCL. This finding is consistent with recent physiological results showing that hilar ectopic granule cells from epileptic rats are more hyperexcitable than granule cells in the GCL.

Theme: Disorders of the nervous system

Topic: Epilepsy: basic mechanisms

Keywords: Hilus; Epilepsy; Biocytin labeling; Asymmetric synapse; Electron microscopy

1. Introduction

The dentate gyrus of the hippocampal formation has three major parts, the hilus, granule cell layer (GCL) and molecular layer [1,12,23,32]. In rats, most granule cells have their cell bodies in the GCL. Their dendrites typically extend into the molecular layer and their axons enter the hilus where they give rise to collaterals and ultimately terminate in stratum lucidum of CA3 [7,8].

Several investigators indicated that granule cell bodies also exist in the hilus [1,14,26,45]. Marti-Subirana et al. [26] described morphological aspects of the ectopic granule-like cells in the albino rat hippocampal formation and classified them into two groups. Using Golgi preparations, they showed that ectopic granule-like cells were distributed throughout the hilar region. The cell body of the monopolar subtype was small (12×9 μm), oval in shape, and had a few occasional somatic spines. One or two primary thick dendrites with spines arose from the cell body. The bipolar granule cell subtype was more infrequent. Its cell body was oval or spherical (13×9 μm). The somata of this subtype had no spines but the dendrites showed numerous spines. An earlier, rudimentary description of misplaced granule cells in the hilus using the Golgi method was made by both Amaral [1] and Seress and Pokorny [45]. In another study, Gaarskjaer and Laurberg

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[14] used a retrograde fluorescent tracing technique to study hilar ectopic granule cells. They determined that 5% of the neurons in the hilus projected to CA3 and that these cells resembled ectopic granule cells. They also showed that the hilar ectopic granule cells had 'dendrites oriented toward' the GCL.

More recent studies showed that hilar ectopic granule cells were increased in epileptic rats. Parent et al. [29] found an increase of dentate granule cell neurogenesis following severe seizures, and described newly generated granule cells in an ectopic location, the hilus. Scharfman et al. [41] observed a large increase in the number of calbindin immunoreactive neurons in the hilus after pilocarpine-induced status epilepticus. Such cells were morphologically similar to granule cells and were suggested to contribute to the hyperexcitability in this model of epilepsy. Electrophysiology of these ectopic granule cells in the hilus from epileptic rats indicated normal electrophysiological characteristics but they also discharged synchronously with spontaneous epileptiform bursts of CA3 pyramidal cells [41]. This abnormal bursting property of hilar ectopic granule cells suggested that they receive mossy fiber input as do the CA3 pyramidal cells.

Previous morphological studies of hilar ectopic granule cells were limited to light microscopic observations. The present study attempts to provide additional morphological data of these cells at the electron microscopic level. Specifically, two issues are addressed. First, do hilar ectopic granule cells have similar features as the typical granule cells in the GCL? Second, what are the synaptic connections of hilar ectopic granule cells? Following biocytin injections into CA3, several retrogradely labeled granule cells were consistently found in the hilus of epileptic and control rats [39]. The goal of the present study is to examine the electron microscopic features of these biocytin-labeled, hilar ectopic granule cells and to determine their synaptic connections.

2. Material and methods

2.1. Induction of status epilepticus

Adult male Sprague–Dawley rats (165–265 g; Zivic-Miller Labs., Allison Park, PA) were used in these studies. The Institutional Animal Care and Use Committees at Duke University and the University of California at Irvine approved animal protocols in advance of the experiments. Ten rats were injected with pilocarpine hydrochloride (300–325 mg/kg, i.p.) preceded 30 min earlier by methylscopolamine (1 mg/kg, i.p.). Six of these animals developed status epilepticus (SE), a continuous limbic motor seizure of stage 2 or higher [31]. SE was terminated after 2.5–4.5 h with a single injection of sodium phenobarbital (50 mg/kg, i.p.). After cessation of SE, animals were maintained as described by Okazaki et al. [27]. Pilocarpine-treated rats that did not develop SE ($n=4$) were not used for this study.

2.2. Granule cell labeling with biocytin

Rats were anesthetized and decapitated 24.9–61.7 weeks after convulsant administration. Brain slices (400 μm thick) obtained from the caudal one-third of the hippocampal formation, transverse to the long axis, were cut with a Vibratome (Technical Products International). They were then transferred to a static interphase chamber (Fine Science Tools) containing oxygenated artificial cerebrospinal fluid (ACSF) at 32.5°C. After a 45-min incubation period to allow for the slice to reach equilibrium in the chamber, a 4% solution of biocytin was iontophoretically ejected into stratum lucidum of CA3b of the hippocampus to label the mossy fibers of dentate granule cells (see Ref. [27]). A glass micropipette with a filament (WPI, 1.5 mm external diameter, 5–10 μm tip diameter) was filled with a freshly prepared solution of 4% biocytin in 0.05 M Tris–HCl buffer, pH 7.3. The tip of the glass micropipette was about 200 μm deep to the surface. Biocytin was ejected into the extracellular space with intermittent positive current pulses (600 nA, 7 s on, 7 s off) for 10–20 min. Three hours following biocytin injection, slices were immersed in a fixative solution containing 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer (PB), pH 7.4, and stored overnight at 4°C.

To visualize the biocytin-labeled granule cells, the fixed slices were re-sectioned at a thickness of 50 μm. The sections were collected in 0.1 M PB and treated with hydrogen peroxide to suppress endogenous peroxidase activity. Then, the sections were washed in 0.1 M PB for 30 min and incubated in avidin–biotin horseradish peroxidase solution (Vectastain Elite ABC Kit, Vector Labs) for 3–5 h at room temperature and overnight at 4°C. Finally, they were washed several times in 0.1 M PB and incubated in 0.025% diaminobenzidine (DAB) and 0.01% nickel ammonium sulfate for 15–20 min.

After the reaction product was formed, the sections were thoroughly washed in PB. Sections were placed in 1% osmium tetroxide for 20–60 min and then processed for embedding in plastic following dehydration through a series of ethanols and immersion in propylene oxide. A flat-embedding procedure was used to facilitate the examination of each section with a light microscope (Leitz). After identifying ectopic granule cells in the hilus, the section was then trimmed using a single-edged razor blade and a dissecting microscope (Nikon). Ultrathin sections containing the dentate gyrus and biocytin-labeled hilar ectopic granule cells were cut with an ultramicrotome (Reichert-Jung) and collected on formvar-coated slot grids. Prior to placing the grids in the microscope, the sections were stained with uranyl acetate and lead citrate to enhance the contrast. Ultrathin sections containing biocytin-labeled, hilar ectopic granule cells were examined

with a Philips CM-10 transmission electron microscope. The hilus, GCL and molecular layer were identified using known morphological features [12]. Then, labeled hilar ectopic granule cells and their synapses were identified and photographed. Axosomatic synapses were counted for both types of granule cells (hilar ectopic and labeled ones in the GCL). The frequency of synapses for labeled cell bodies and proximal dendrites was expressed as the number per unit length of membrane for a single thin section.

3. Results

For this analysis 12 biocytin-labeled hilar ectopic granule cells were examined from four of the six epileptic rats. The light microscopic examination of biocytin-labeled preparations from rats with SE showed brown reaction product in many cell bodies in the GCL and in adjacent processes, such as the dendrites in the molecular layer and mossy fibers in the hilus and area CA3 (Fig. 1A). In addition, these preparations contained biocytin-labeled, hilar ectopic granule cells that were identified by their location in the hilus and by the presence of retrograde labeling for biocytin throughout their somata and processes. They were found either close to the GCL (Fig. 1B) or in the deep hilus (Fig. 1A) and bordering the pyramidal cell layer in area CA3c (see Ref. [41]). The cells that were chosen for electron microscopic examination were located 20–40 μm from the GCL (Fig. 1B) because this site allowed for a direct comparison with the granule cells in the GCL. The hilar ectopic granule cells were usually bipolar with thick dendrites extending from each end of the cell body. The dendrites of these cells showed numerous spines and they often penetrated the GCL and arborized within this layer but could not be followed into the molecular layer. The long axis of the hilar ectopic granule cells examined in this study was perpendicular to the GCL (Fig. 1B)

Electron microscopic preparations of these same specimens showed many biocytin-labeled and unlabeled granule cells in the GCL and a few biocytin-labeled ectopic granule cells in the hilus where they were surrounded by neuropil (Fig. 2). The labeled granule cells had electron dense reaction product in their perikaryon and dendritic cytoplasm (Fig. 2). In general, the somata of hilar ectopic granule cells had similar nucleoplasmic and cytoplasmic features as granule cells in the GCL. A brief description is given below of biocytin-labeled granule cells in the GCL followed by a description of the features of hilar ectopic granule cells.

3.1. Ultrastructural features of typical granule cells

Biocytin-labeled granule cell bodies are common in the GCL (Fig. 2) and have ultrastructural features that are similar to those previously described for dentate granule cells [4,21,33]. These typical cell bodies have a diameter of approximately 10 μm and are either round or oval-shaped. The oval-shaped cell bodies were commonly found near the hilar border and had their long axis perpendicular to the GCL (Fig. 2). Most of the cell body of typical granule cells is occupied by the nucleus that displays several chromatin aggregates and has a relatively smooth surface. The perikaryon is limited to a thin shell around the nucleus and this part of biocytin-labeled granule cells contained electron dense reaction product. A few cisternae of the granular endoplasmic reticulum and free ribosomes are found in the perikaryon but they are not aggregated together to form Nissl bodies, as they are in the large pyramidal neurons of CA3. In addition, numerous mitochondria, free ribosomes, lysosomes and Golgi apparatus are present (Fig. 2). Dendrites were observed to arise from the apical pole of granule cells (Fig. 2). They were typically 2–3 μm in diameter at their origin. A thin axon initial segment was found to emanate from the basal pole.

3.2. Ultrastructural features of hilar ectopic granule cells

The hilar ectopic granule cells have ultrastructural features that are similar to those of granule cells in the GCL with some morphological differences. The size of the cell body of these cells was in the same range as that for typical granule cells. The somata were round or oval shaped and located in the hilus with their long axis perpendicular to the GCL (Fig. 2). The perikaryon of hilar ectopic granule cells was similar to that for the granule cells in the GCL and contained the same cytoplasmic organelles (Figs. 3A and 4). On the other hand, several features were different than those for typical granule cells. The nuclei of hilar ectopic granule cells often displayed infoldings or were notched as opposed to being round or oval (Figs. 3A and 4A). Also, the proximal portions of hilar ectopic granule cell dendrites were larger in diameter than those of typical granule cells in the GCL (Fig. 3B,C). At their origin, these thicker dendrites were 4–6 μm in diameter, and they tapered down to about 2 μm at the hilar/GCL border. Similar to typical granule cells, these ectopic granule cells had numerous spines on their dendrites (Figs. 3C and 5). The axon initial segments of granule cells were not observed in the electron microscopic preparations because a complete serial section analysis was not made of these cells.

3.3. Synaptic connections of hilar ectopic granule cells

We examined five labeled somata of hilar ectopic granule cells for axosomatic synapses. In this analysis, asymmetric (presumed excitatory) and symmetric (presumed inhibitory) synapses were distinguished using established criteria for hippocampal synapses [12,21,38,42]. Unlabeled axon terminals formed exclusively asymmetric

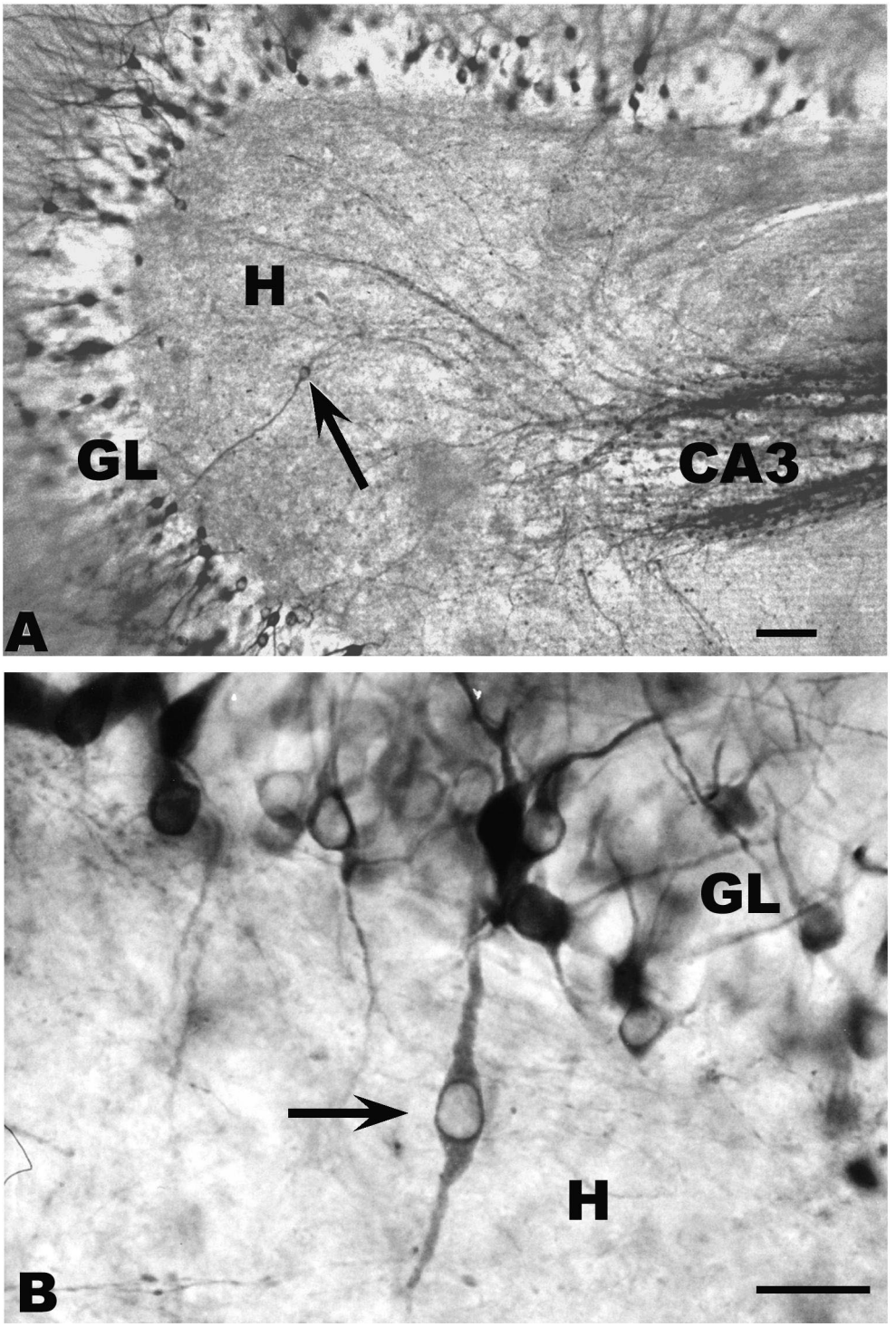

Fig. 1. Photomicrographs of biocytin-labeled granule cells. (A) A low magnification of the granule cell layer (GL) containing biocytin-labeled cells and the large bundle of mossy fibers in CA3. An ectopic biocytin-labeled granule cell (arrow) is shown in the hilus (H) with a long dendrite extending into the granule cell layer. (B) An enlargement of another biocytin-labeled ectopic granule cell in the hilus (H). Note the thick dendrite extending toward the GL and its long axis that is perpendicular to the hilar border with the GL. Scale bars: 50 μm in (A) and 25 μm in (B).

synapses with the somata of hilar ectopic granule cells (Fig. 4B,C). Many of the unlabeled axon terminals were large (1–2 μm), contained clear round synaptic vesicles spread throughout the terminal, and had occasional dense-core vesicles and mitochondria that were located away from the active synaptic sites (Fig. 4). No biocytin-labeled axon terminals formed axosomatic synapses with hilar ectopic granule cells. A total of 32 axosomatic synapses

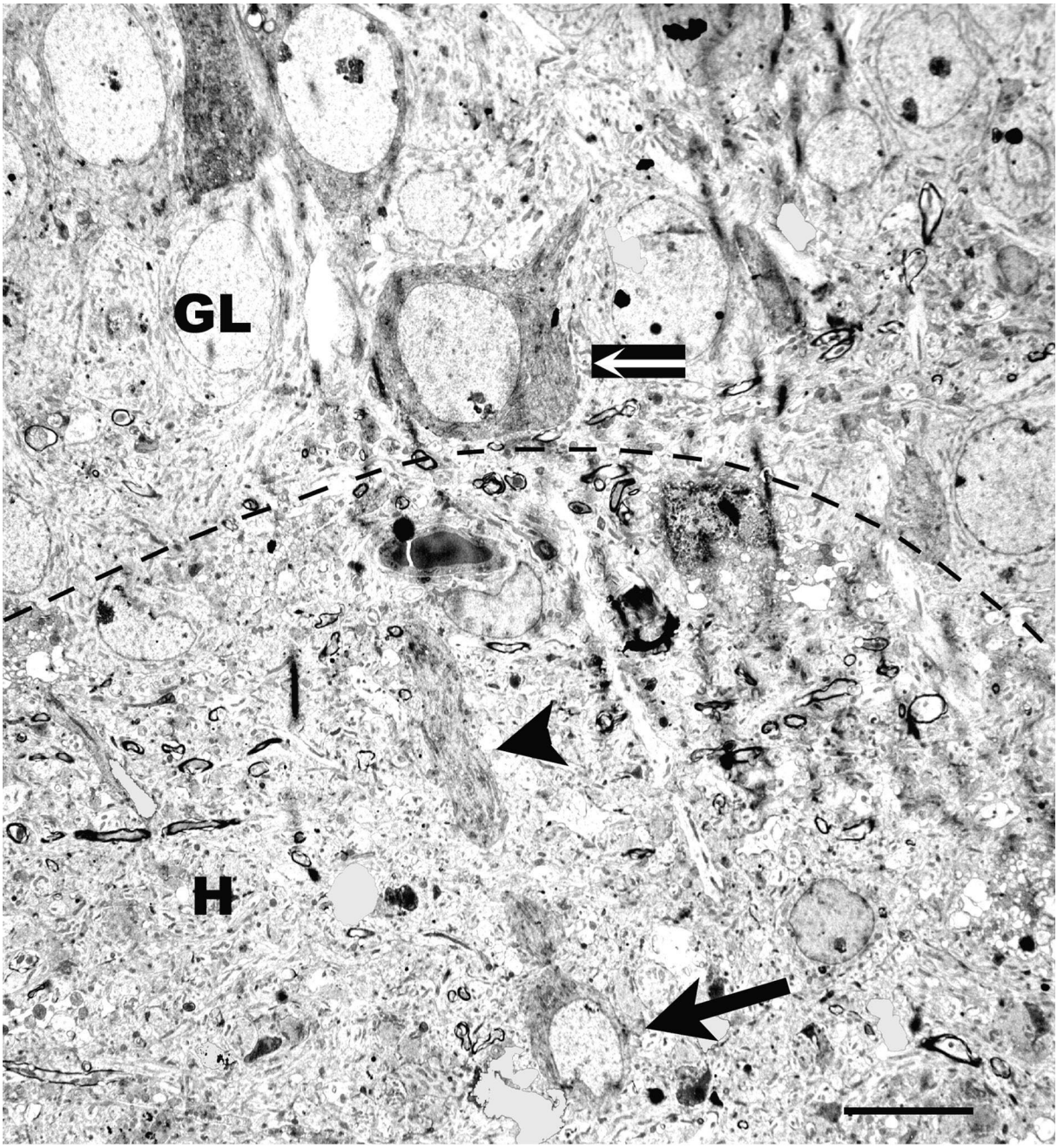

Fig. 2. Electron micrograph of a biocytin-labeled ectopic granule cell (arrow) and its dendrite (arrowhead) in the hilus (H). The position of this hilar ectopic granule cell is about 30 μm below the granule cell layer (GL). Note that the GL contains both labeled (white arrow) and unlabeled granule cells. The dashed line shows the border between the GL and H. Scale bar: 10 μm.

were found for 149 μm length of somal membrane for the five examined cells. Thus, the mean number of axosomatic synapses was 6.4 per soma per thin section. In contrast, labeled granule cells ($n=5$) in the GCL had significantly fewer axosomatic synapses per soma per thin section (mean number=2.0; $P<0.05$, Student's *t*-test).

Labeled dendrites of hilar ectopic granule cells were also examined for synaptic connections. The thin sections facilitated an analysis of only the proximal portions of the dendrites that were found in the hilus. Dendrites were not examined in the GCL. Unlabeled axon terminals with a similar morphology as that described above for axosomatic synapses also formed asymmetric synapses with the *dendrites* of all the examined hilar ectopic granule cells (Fig. 5). It should be noted that *biocytin-labeled* axon terminals also formed asymmetric synapses with dendrites and spines of hilar ectopic granule cells (Fig. 5A). These axon terminals had the same morphology and size as those in previous studies that identified them as mossy fibers [27,39]. The frequency of axodendritic synapses was

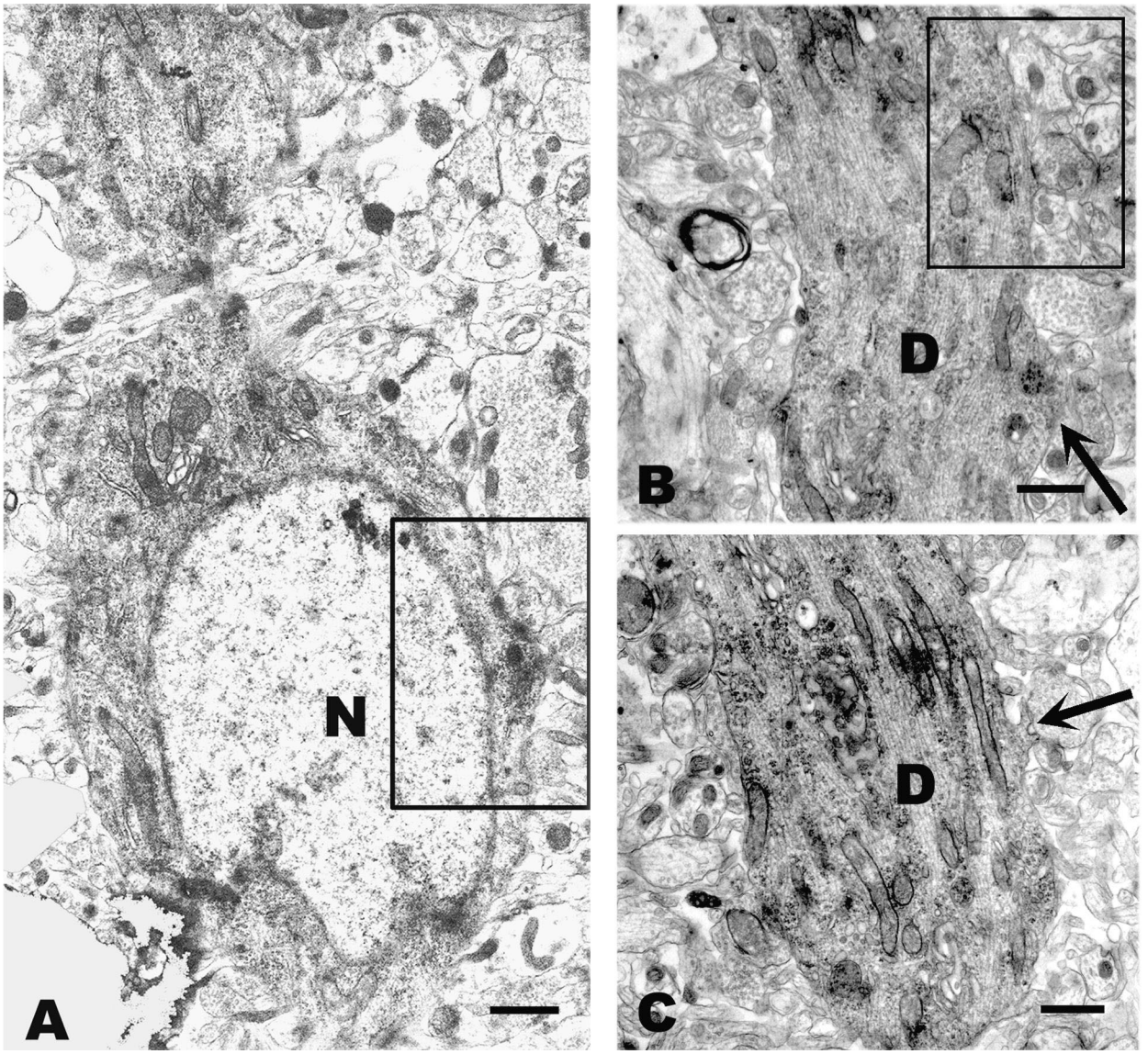

Fig. 3. Higher magnifications of the soma and dendrite of the hilar ectopic granule cell in Fig. 2. (A) The enlargement of the soma of this hilar ectopic granule cell. The ovoid shaped cell body is occupied by a large nucleus (N) that displays a notch in its lower left region. The perikaryon is confined to a thin shell with several cytoplasmic organelles such as endoplasmic reticulum, ribosomes, few mitochondria, lysosomes and Golgi apparatus. (B) An enlargement of this labeled cell's hilar dendrite (D) with a stubby spine (arrow). Note the cytoplasmic organelles in the dendrite. (C) An enlargement of another portion of this cell's dendrite (D) that bears a mushroom-shaped spine (arrow). The boxed areas are enlarged in subsequent figures. Scale bars: 1 μm for (A) and (B).

significantly greater than that of axosomatic synapses (one per 3.08 μm for axodendritic versus one per 4.65 μm for axosomatic; $P<0.05$, Student's *t*-test).

4. Discussion

This study has shown the electron microscopic features of retrogradely labeled hilar cells following biocytin injections into CA3b and indicates that they are ectopic granule cells. Furthermore, these hilar ectopic granule cells have three times more total synapses on their somata than granule cells in the GCL. Because ectopic granule cells have mainly asymmetric synapses on their somata and proximal dendrites, this finding suggests that they are more hyperexcitable than granule cells in the GCL.

4.1. Technical considerations

The injection of biocytin is an effective method for labeling both dendritic and axonal processes of neurons [22,27]. In the present study, biocytin was ejected into stratum lucidum of CA3b of the hippocampus to target the mossy fiber projection system of dentate granule cells. In so doing, many granule cells in the GCL are retrogradely labeled [27,39]. Biocytin was chosen for our study because

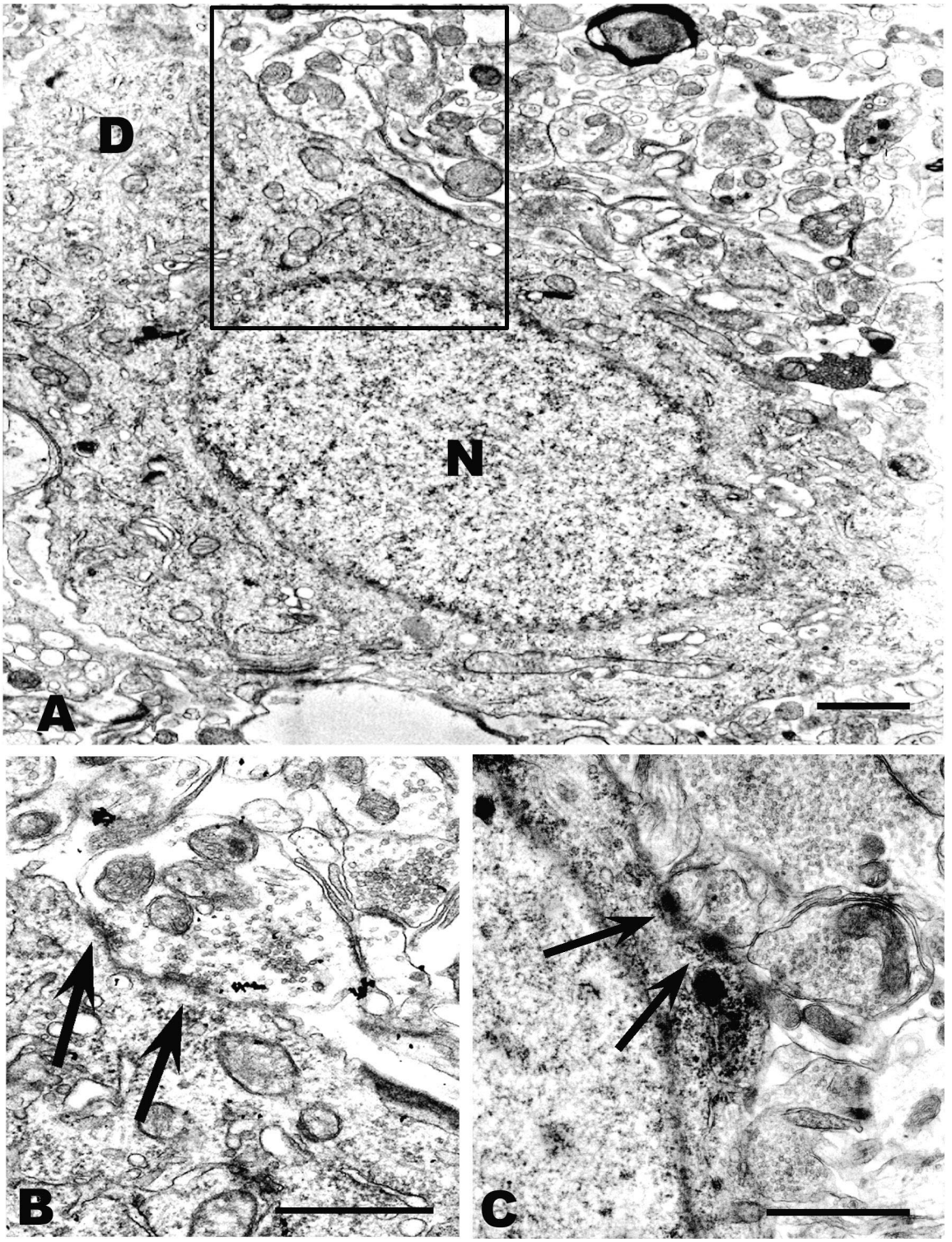

Fig. 4. Electron micrographs of another example of a biocytin-labeled hilar ectopic granule cell and axosomatic synapses of this cell and the one in Fig. 3. (A) Another hilar ectopic granule cell with the same ultrastructural morphology shown for the cell in Fig. 3. Note the thick proximal dendrite (D) and notched nucleus (N). (B) An enlargement of an asymmetric axosomatic synapse (arrows) on the hilar ectopic granule cell in A (see box in A). (C) Another example of an asymmetric axosomatic synapse (arrows) from the hilar ectopic granule cell in Fig. 3A (see box). Both synapses display two active sites. Scale bars: 1 μm for (A–C).

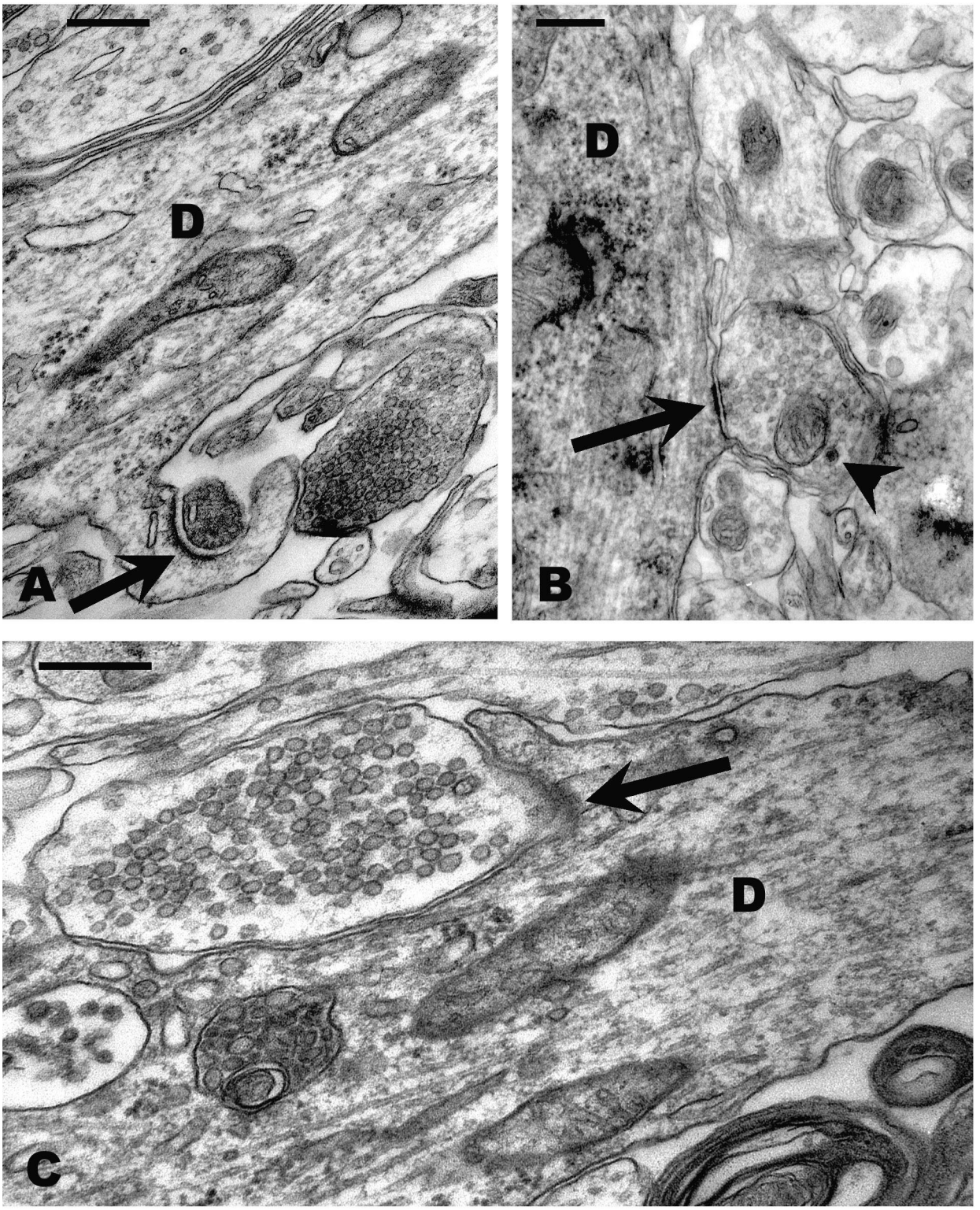

Fig. 5. Electron micrographs of axodendritic synapses for biocytin-labeled, hilar ectopic granule cells. (A) A biocytin-labeled axon terminal and its asymmetric synapse (arrow) with a dendritic spine arising from a dendrite (D) of a hilar ectopic granule cell. Note the thickened postsynaptic density in the spine (arrow). (B) An enlargement of the synapse in Fig. 3B (see box). It shows an example of an asymmetric synapse (arrow) between a dendrite (D) of a hilar ectopic granule cell and an unlabeled axon terminal. A dense core vesicle (arrowhead) is present in the unlabeled axon terminal and together with the asymmetric synapse suggests that this synapse is formed by a mossy fiber (see text). (C) Another example of an asymmetric synapse with a dendritic spine (arrow) of a biocytin-labeled hilar ectopic granule cell. Note that the labeled dendrite (D) displays greater electron density than the unlabeled axons in this panel. Scale bars: 0.3 μm for (A–C).

it selectively labels the neurons with axons projecting to the injection site, is a non-fluorescent tracer that can be visualized with the electron microscope, and does not leak out of membranes at synapses. The light microscopic results from this study and a previous one from this laboratory [39] demonstrated hilar ectopic granule cells, and were consistent with the previous results of Gaarskjaer and Laurberg [14] who used a *fluorescent* retrograde labeling method. In these studies, retrograde labeling was mainly limited to granule cells, including those in the GCL and ectopic ones in both the hilus and molecular layer.

4.2. Identification of biocytin-labeled hilar cells

Are the biocytin-labeled cells in the hilus ectopic granule cells or another type of hilar neuron as described by Amaral [1]? Several reasons indicate that the biocytin-labeled cells in the hilus are displaced granule cells and they will be discussed below.

First, biocytin is not transferred through the membrane of labeled neurons, so biocytin-labeled cells in the hilus must have axonal projections to stratum lucidum in the ipsilateral CA3 region of Ammon's horn. Neurons with this projection are thought to be exclusively granule cells [1,14,23,32]. Second, in addition to this observation which identifies the biocytin-labeled cells in the hilus as granule cells, ultrastructural features of these ectopic cells show several morphological features that are characteristic of granule cells. These include: a soma with a diameter of about 10 μm that is mainly occupied by a nucleus, a thin perikaryon with several organelles but no Nissl bodies, and a nucleus with several heterochromatic regions. In addition, the dendrites are orientated orthogonal to the GCL and have many spines. These ultrastructural features are the same as those described previously for granule cells in the GCL [4,21,33]. Third, in the following paragraphs, the known ultrastructural features of a limited number of hilar neurons are presented to show that the biocytin-labeled hilar cells in the present study can be excluded as one of these hilar cell types.

The most common cell type in the hilus, and certainly the most impressive in size and structure, is the mossy cell [1,37]. The mossy cell's ultrastructural features include a large soma (20–30 μm diameter) with a round or oval, euchromatic nucleus that lacks infoldings and intranuclear rods [5,13,25,37,49]. The perikaryon of mossy cells contains organelles typical of comparably sized pyramidal cell bodies, including Nissl bodies. The mossy cell bodies also display numerous somal spines. Most of these are complex in shape and may branch into three or four smaller spines. The dendrites arise from the cell bodies of mossy cells as large thick tapering structures and have numerous complex spines or thorny excrescences on their proximal portions. These features were not observed for any of the biocytin-labeled cells located in the hilus in the present study.

Two types of fusiform cells in the hilus also were characterized at the electron microscopic level [36]. The fusiform cells of the dentate gyrus are located in a portion of the hilus within 100 μm of the GCL and have ovoid somata and bipolar dendrites with a long axis that is *parallel* to the border between the hilus and GCL [36]. These neurons are distinguished by their dendrites: either spiny or sparsely spiny varieties. The spiny type of fusiform neuron has a round or ovoid nucleus that lacks infoldings and intranuclear rods, and occupies more than half of the somal area. The spiny fusiform cell has numerous spines along its dendrites. This cell type also displays somal spines that occasionally have mitochondria within their heads. In contrast, the sparsely spiny fusiform cell displays only a few spines. This cell has a highly infolded, often eccentrically located nucleus. A prominent nucleolus is also observed as well as intranuclear rods. Once again, these features are not observed for any of the biocytin-labeled cells located in the hilus in the present study. Therefore, the biocytin-labeled hilar cells in the present study are not fusiform cells. These data taken together with the other features described above allow us to conclude that the biocytin-labeled cells are most likely hilar ectopic granule cells and not one of the other major cell types observed in the hilus of the dentate gyrus.

4.3. Connections of hilar ectopic granule cells

In addition to the analysis of the cytological features of ectopic granule cells, the present study analyzed their synaptic connections. Axon terminals presynaptic to the somata and proximal dendrites of labeled hilar ectopic granule cells formed exclusively asymmetric synapses. The fact that some of these axon terminals were labeled (Fig. 5A) indicates that mossy fibers are forming synapses with hilar ectopic granule cells. This finding suggests that granule cells target hilar ectopic granule cells and may provide the basis for increased excitatory input to them.

The absence of symmetric synapses on the somata and proximal dendrites of hilar ectopic granule cells indicates that the inhibitory basket cell input to a portion of these granule cells is missing. Pyramidal basket cells have an axonal plexus in the GCL that is responsible for the formation of symmetric synapses with the somata and proximal dendrites of granule cells in the GCL [12,15,35,44]. The pyramidal basket cell axon arises from the apical dendrite and ramifies in the inner molecular layer before dropping vertical axon collaterals into the GCL [32]. Therefore, it is unlikely that the axons of pyramidal basket cells will synapse with the cell bodies of hilar ectopic granule cells because they lie outside the GCL. The location of the soma and lack of symmetric axosomatic synapses seen in the present study suggests that hilar ectopic granule cells lack inhibitory input on their somata and proximal dendrites. It should also be noted that granule cells in the GCL have a lower mean number of axosomatic synapses than hilar ectopic granule

cells. The data for axosomatic synapses per granule cell in the GCL in the present study (mean=2.0) was similar to that shown previously by Seress and Ribak [46] where 205 granule cells in the GCL had a mean of 2.2 synapses per soma per thin section.

4.4. Functional significance of morphological findings

A unique ultrastructural feature of the hilar ectopic granule cells is a notched or ruffled nucleus. Typical granule cells in the GCL do not display this morphology [4,21,33]. What is the meaning of this type of nuclear morphology? Previous studies have shown that infolded nuclei are characteristic of high levels of neuronal activity [33,43]. This notching or ruffling of the nucleus increases the surface area of the nuclear membrane, and allows for increased transfer of RNA in these cells. Thus, notched nuclei in hilar ectopic granule cells indicate a high level of activity for these cells, which may include hyperexcitability and a role in hippocampal seizures [41].

The granule cells of the adult hippocampal dentate gyrus are now considered to be a dynamic population of neurons that undergo neurogenesis throughout the adult mammalian life [2,6,11,17–20]. Parent et al. [29] and Bengzon et al. [3] showed increased numbers of mitotically active cells below the GCL differentiating into neurons with preparations that labeled bromodeoxy-uridine (BrdU) sites of incorporation into DNA and the early postmitotic marker, TOAD-64. They hypothesized that differentiation of a population of newly born granule cells, rather than remodeling of mature granule cells, is the basis for the network reorganization seen in some forms of human temporal lobe epilepsy. More recently, Scharfman et al. [41] showed increased numbers of calbindin immunoreactive granule-like cells in the deep hilus after pilocarpine-induced status epilepticus and suggested that newly born or previously existing granule cells migrate to this location. They also indicated that these ectopic cells contribute to the hyperexcitability present in this model and may participate in the generation of spontaneous seizure activity because they display synchronous bursting behavior with CA3 pyramidal cells. The present anatomical data are consistent with these findings. Analysis of synaptic connections indicates a predominance of excitatory inputs, including biocytin-labeled mossy fiber input, and confirms the speculation (see Section 1) that ectopic granule cells in the hilus are targeted by mossy fibers that predominate in this region.

Some of our findings could have important implications for understanding the mechanism of seizure propagation in the epileptic brain. The dentate gyrus is thought to function as a buffer against seizure propagation through the limbic circuit [9,24]. Dentate granule cells resist seizure propagation for several reasons, including strong tonic inhibition from GABA interneurons [12,28] and lack of a synaptic mechanism for synchronization of cell discharge. With respect to the latter point, there are few synaptic connections among granule cells in the GCL [34,40] and few hilar ectopic granule cells in the normal brain [14,39]. In pilocarpine-treated epileptic rats, however, the numbers of both increase dramatically [41]. Hilar ectopic granule cells become integrated into the circuitry of the dentate gyrus, such that they receive excitatory innervation from other granule cells (present study) and send axon collaterals into the hilus and area CA3 [41]. Furthermore, hilar ectopic granule cells in the epileptic brain differ from granule cells in the GCL in that they exhibit spontaneous bursts that are time-locked to spontaneous population bursts of CA3 pyramidal cells [41]. Their propensity for burst discharge may be related to the paucity of inhibitory synapses on the somata and proximal dendrites of hilar ectopic granule cells. Even in the epileptic brain, hilar ectopic granule cells account for only a small percentage of the total granule cell population. However, Traub et al. [48] showed that the synchronous discharge of only a few CA3 pyramidal cells in the normal brain could trigger a population burst because these neurons are synaptically interconnected. By analogy, bursting of the relatively few hilar ectopic granule cells may recruit the normal granule cell population into epileptiform activity via their reciprocal synaptic connections. In this way, hilar ectopic granule cells could be critical for the synaptically driven reverberating excitation characteristic of the dentate gyrus in the epileptic, but not in the normal, brain [10,16,30,47].

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